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Previews

Shigella Stands up to the Challenge of Adhesion

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The invasion process of *S. flexneri* is well characterized, but mechanisms underlying this bacterium's adhesion to host cells have remained obscure. In this issue of *Cell Host & Microbe*, Brotcke Zumsteg et al. (2014) report a surprising role for the *Shigella* virulence factor IcsA (VirG) as an adhesin.

Shigella flexneri is a model pathogen. It has been used extensively to study type III secretion, a cytosolic pathogenic lifestyle, cytosolic immune surveillance, and actin polymerization. In each of these aspects, *S. flexneri* has contributed to significant advances in cellular microbiology. However, in order for all of these intracellular steps to occur, the bacterium must first make contact with host cells. Mechanistic insight into *S. flexneri* adherence to host cells has eluded researchers for decades. *Shigella* was missing an important virulence factor: an adhesin.

In a landmark discovery in the 1980s, the protein IcsA (VirG) was shown to mediate intercellular spread (Makino et al., 1986; Bernardini et al., 1989). IcsA localizes to the outer membrane in an asymmetric manner, accumulating at one bacterial pole. By recruiting host cell actin via N-WASP, IcsA is able to create a comet tail that thrusts the bacterium into neighboring host cells (Figure 1). Slightly later, the discovery of the MxiSpa type III secretion system (T3SS) facilitated our understanding of the invasion process and highlighted the role of IpaB and IpaD proteins as major invasins. However, until recently, it was not known that IcsA and the T3SS influence each other.

To identify adhesins in *S. flexneri*, Brotcke Zumsteg et al. (2014) started with an old but astute observation that *Shigella* strain lacking IpaB or IpaD is hyperadhesive (Ménard et al., 1994). Here, the authors confirmed the hyperadhesive phenotype of the two mutants in a variety of cells lines. Surprisingly, scanning electron microscopy (SEM) analysis showed that the hyperadhesive mutants predominantly contacted macrophages and neutrophils using one pole, whereas the wild-type strain tended to contact cells using its long axis. This clue led to the remarkable hypothesis that hyperadhesion to host cells is mediated by one or more molecules with a polar localization.

Because IcsA localizes to one pole, the authors tested whether it contributed to hyperadhesion. First, they showed that deleting *icsA* abrogated hyperadhesion of the *ΔipaD* mutant and at the same time abolished polar adhesion to host cells. To further investigate their finding, the authors produced IcsA in a *Shigella* strain cured of the virulence plasmid (BS176). As both IcsA and the T3SS are encoded on the large virulence plasmid, this experiment allowed the authors to uncouple these two virulence factors. IcsA alone was not sufficient to induce adhesion in BS176, and they concluded from this experiment that an activation step is required for IcsA-dependent adhesion in *Shigella*. What is the activation step? Strains lacking the *ipaD* gene are constitutively active for T3SS expression, leading the authors to logically conclude that the adhesion capacity of IcsA is somehow triggered upon T3SS activation (Figure 1). In line with this hypothesis, treatment with the bile salt deoxycholate (DOC), a stimulus encountered by the bacterium in the host intestine, induced IcsA-dependent adhesion. Importantly, DOC induced adhesion only when the bacterium had an intact T3SS.

Next, they asked whether IcsA is sufficient to promote adhesion to host cells in the absence of the T3SS. To test this, IcsA was expressed in *E. coli*. Paradoxically, and in contrast to plasmid-cured *Shigella*, when IcsA was expressed in *E. coli*, the bacterium adhered using IcsA in the absence of an activation step. While this reductionist experiment demonstrates sufficiency of IcsA for adhesion, the dissonant results between *E. coli* and BS176 led the authors to question whether IcsA might exist in different conformations, only one of which was adhesive. Indeed, the authors found that when IcsA functioned as an adhesin, it had an altered protease cleavage pattern compared to IcsA in non-adhering strains. These intriguing results will hopefully spur an in-depth biochemical analysis of IcsA to substantiate the findings.

These data suggested that IcsA plays a dual role during shigellosis by promoting both adhesion to host cells and intercellular dissemination. To uncouple these two functions, the authors screened an IcsA mutant library for clones that were unable to adhere but could still form plaques. The successful identification of this mutant then allowed the authors to test in vivo whether IcsA-dependent adhesion contributed to pathogenesis. Indeed, an adhesion-deficient IcsA mutant was attenuated in a mouse model of shigellosis, cementing the role of IcsA as an adhesin in *Shigella*.

While the work described answers the long-standing question of what the adhesin in *Shigella* is, it raises many more interesting questions that call for more exploration. What sort of signaling cascade connects the T3SS and IcsA? The authors rule out MxiE and IpgC, two previously described transcriptional regulators that are active only upon T3S activation. This suggests that an as-yet-undescribed pathway might also be at play. Does IcsA really exist in alternate conformations? Or perhaps binding to another factor or posttranslational modifications leads to altered protease accessibility. These possibilities will require further investigation.

Another emerging question asks what the host cell receptor for IcsA is. Interestingly, IcsA-dependent adhesion does not

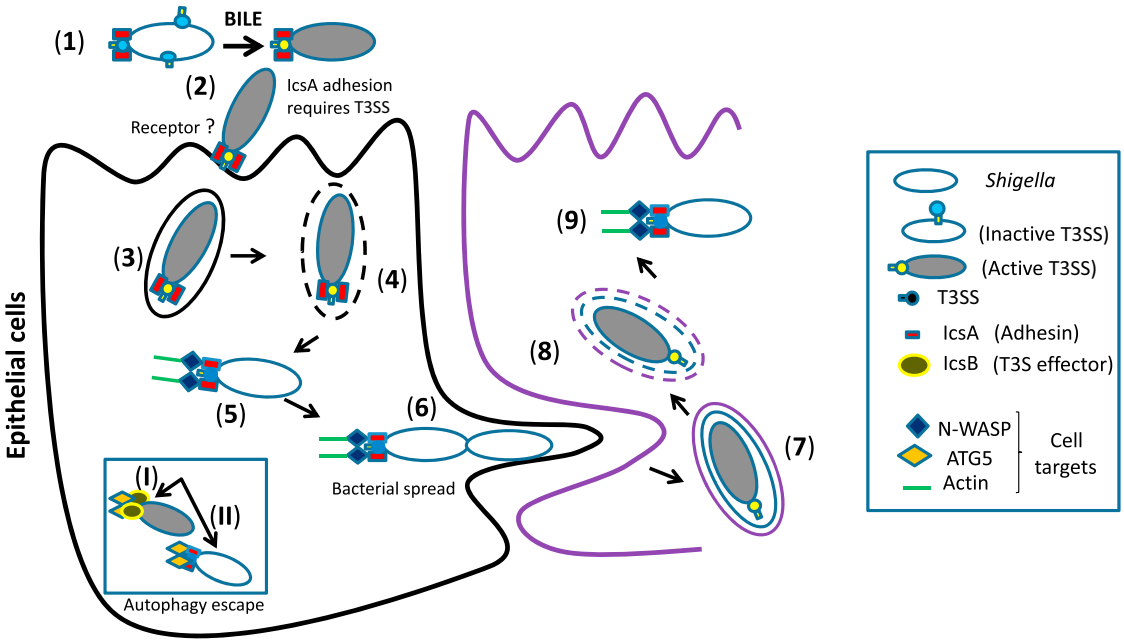

Figure 1. Model Summarizing the Crosstalk between the T3SS and the Dual Role of IcsA in Cell Adhesion and Bacterial Spread
Treatment with the bile salt deoxycholate (DOC), a stimulus encountered by the bacterium in the intestine, induces polar activation of the T3SS to produce an adhesive form of IcsA (1). IcsA acts as an adhesin by binding to an as-yet-unidentified receptor (2). After bacterial entry, an active T3SS is required for vacuole lysis (3 and 4). Once free within the cytoplasm, *Shigella* uses IcsA to bind to N-WASP to recruit and induce actin polymerization at one bacterial pole (5) (Egile et al., 1999). The unilateral movement allows bacterial passage to neighboring adjacent cells (6), a step during which the T3SS is switched off. Next, bacteria lyse the two cell membranes acquired during protrusion (7 and 8), a step requiring an activated T3SS. Lastly, free bacteria will restart a new infection process (9). (I) *Shigella* escapes killing by autophagy upon IcsB binding to ATG5 (Ogawa et al., 2005). (II) In the *icsB* mutant background, IcsA binds to ATG5 to facilitate bacterial killing by autophagy (Ogawa et al., 2005).

appear to answer why shigellosis is restricted to humans, since IcsA contributed to adhesion of mouse and human cells. Likewise, IcsA-dependent adhesion did not show specificity for any cell type. All previously identified IcsA interaction partners in the host have a cytosolic localization (Figure 1) (Suzuki et al., 1998, Egile et al., 1999, Ogawa et al., 2005), suggesting that finding the IcsA receptor will require an unbiased fishing approach with extracellular-exposed molecules.

The study also has important implications for vaccine design, an area that has challenged *Shigella* researchers because of the sheer diversity of serotypes. Several vaccine candidates have relied on *icsA* mutations to attenuate the bacterium for live, oral delivery. Taking these new data into consideration, this approach now appears to have two shortcomings. First, the *icsA* mutant is not likely to adhere to the intestines (Rahman et al., 2011), a prerequisite for inducing immunity. Second, the vaccine would never elicit antibodies against IcsA if IcsA is missing. Since IcsA is a conserved virulence factor and an adhesin, it serves as an excellent target for inducing broadly neutralizing *Shigella* antibodies. In this context, several works reported that IpaD subunits induce antibodies that are able to neutralize invasion by *Shigella* of several serotypes. Perhaps combining these strategies would provide the immunogenicity necessary to prevent shigellosis.

Another interesting concept raised by the paper is the idea of crosstalk between the T3SS and IcsA. Other studies have hinted at this; one showed that the T3S effector IpaC accumulates at the same pole as IcsA prior to secretion (Jaumouillé et al., 2008). If there is indeed coordination between these disparate virulence strategies, it could mean that the bacterium is primed to deliver T3S effectors to the pole adhering to host cells. Likewise, Campbell-Valois et al. (2014) presented an additional link between the T3SS and IcsA in a recent issue of *Cell Host & Microbe*. The authors developed a fluorescent reporter to monitor activity of the T3SS during invasion and intercellular spread. The T3SS showed two spikes in activity, during initial invasion of the bacterium and during intercellular spread, when the double membrane was degraded (Figure 1). If *Shigella* was unable to create actin comet tails, either because of an *icsA* deletion or by interfering with actin polymerization, the bacterium could not reactivate the T3SS. This suggests that the crosstalk goes in both directions, where the T3SS is required for activating adhesion through IcsA, but that IcsA is also required for activation of the T3SS in some instances. Future experiments in the *Shigella* field will require attentiveness to this emerging dialog.

Last but not least, the retrieved lesson from this study is to highly consider the physiological environmental conditions when studying the virulence process. The paper of Brotcke Zumsteg et al. (2014) will certainly open new research avenues, including the revisiting of some old accepted concepts.

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A Host MicroRNA Brokers Truce with HSV-1

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Establishing lifelong infection and periodically shedding infectious progeny is a successful strategy employed by several persistent pathogens. In this issue of *Cell Host & Microbe*, Pan et al. (2014) demonstrate that a cell-type-specific host microRNA can restrict gene expression and pathogenicity of herpes simplex virus 1, thereby promoting long-term infection.

In any prolonged war, détente, *a long-term relaxation of strained relations or tensions between two nations*, represents a desirable outcome. This also applies for the ongoing war between host and pathogen, and particularly so for those pathogens that have long coevolved with their hosts. For a virus, establishing lifelong infection maximizes the opportunity to spread to more hosts, thereby providing an evolutionary advantage. Viruses have evolved a number of strategies for persistent infection, and arguably the most elegant is latency. Latent viruses can undergo a lytic growth phase, involving replication of the viral genome, production of progeny virus, lysis of cells, infection of neighboring cells, and spread to other organisms. During this phase, the virus also infects cells within the host where it is able to establish latency. Latency results in drastically reduced expression of most viral genes and eliminates the production of infectious progeny, allowing the virus to evade the immune response. Periodically, the virus reactivates from this quiescent state in response to a variety of environmental cues and re-enters the lytic phase of its life cycle.

Members of the *Herpesviridae* family could be considered the champions of latent infection. There are several human pathogens in this family, including Epstein-Barr virus, human cytomegalovirus (HCMV), varicella-zoster virus, and herpes simplex virus type 1 (HSV-1). HSV-1 replicates primarily in epithelial cells but establishes latency in neural ganglia. During latency HSV-1 limits gene expression to a single locus, the non-protein-coding latency-associated transcript (LAT), and coordinately reduces lytic gene expression to near-undetectable levels. Both viral and host factors are believed to be involved in establishing and maintaining latency in neuronal cells, but the mechanisms are still poorly understood.

In one sense, the latent phase of the viral life cycle is simply the repression of the lytic phase. In herpesviruses, the immediate early (IE) genes drive initiation of the lytic phase. In HSV-1, the IE viral protein ICP0 is required for reactivation (Boutell and Everett, 2013) and for controlling its own expression as well as other IE genes that are critical to maintaining latency. MicroRNA (miRNA)-mediated silencing has garnered considerable attention as a potential mechanism for this regulation during latency (Cullen, 2011). Consistent with this hypothesis, members of both the *Herpesviridae* and *Polyomaviridae* families encode autoregulatory miRNAs that control expression of their lytic genes. For example, the LAT locus of HSV-1 encodes several miRNAs (Umbach et al., 2008; Jurak et al., 2011), which have been linked to downregulation of the IE genes ICP0 and ICP34.5 (Umbach et al., 2008). Thus, it is firmly established that diverse viruses can use miRNAs to regulate the expression of their own genes—a potential factor in both establishing and maintaining latent infections.

miRNA regulation is prevalent in multicellular eukaryotes and, directly or indirectly, likely regulates all cellular pathways. miRNAs are central components of the RNA-induced silencing complex (RISC), targeting it to messenger RNAs (mRNAs) via base pairing. Numerous miRNAs have cell-type-specific expression, making them attractive candidates as host factors involved in circumstantial regulation of lytic gene expression. Indeed, there are examples of host miRNAs affecting the cell-type-specific gene expression of diverse viruses (Gunasekharan and Laimins, 2013; Trobaugh et al., 2014; Jopling et al., 2005). Despite what is known, many questions remain regarding the extent to which host miRNAs are coopted or